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The *soxRS* System of *E. coli* and *Salmonella*: at the Junction of Oxidative Stress and Antibiotic Resistance

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All cells have the ability to respond to increased levels of reactive derivatives of oxygen or nitrogen, so-called oxidative stress. There are different forms of oxidative stress, such as elevated hydrogen peroxide (e.g., from leaks in the respiratory chain), elevated superoxide (e.g., due to redox-cycling compounds, such as paraquat), or elevated nitric oxide (primarily through the activity of inflammation-associated nitric oxide synthase). The responses to these different types of free radical stress vary. In *E. coli*, the response to superoxide stress is regulated primarily by the *soxRS* system. The oxidation of [2Fe-2S] centers in the SoxR protein activates it to stimulate transcription of the *soxS* gene. The increased level of SoxS protein then increases transcription of the $\geq$65 genes of the *soxRS* regulon, through the recruitment of RNA polymerase to the promoters. In cells not subjected to oxidative stress, the iron-sulfur centers of SoxR are maintained in the reduced state by cellular activities.

Later, we discovered that SoxR can also be activated in cells exposed to nitric oxide. The activation by NO is fundamentally different than the superoxide response: SoxR is activated by direct reaction of NO with the [2Fe-2S] centers, which leads to their partial destruction and the formation of dinitrosyl-iron-dithiol complexes in the protein. These are stable enough in vitro that the nitrosylated protein can be purified to demonstrate its transcriptional activity. However, in the cell the nitrosylated complexes are turned over rapidly, which indicates an active process to dispose of them.

The mechanism by which the redox and nitrosylation signals in SoxR are transmitted into gene activation remains a question of great interest. SoxR activates *soxS* transcription by modulating the promoter structure, and structural studies of related proteins support this view. For SoxR, while awaiting detailed structural information, we have approached this problem by partial proteolysis to map redox-induced changes in protein structure, and by genetic approaches to separate the DNA binding and signal transduction functions. We have isolated a large number of mutant forms of SoxR that retain the ability to bind the *soxS* promoter, but which no longer activate transcription. These mutations map to regions around the [2Fe-2S] centers, to a long helical region that likely forms a dimer interface, and even to the DNA binding domain itself. Analysis of these alterations in the context of the structural information suggests that a coordinate transition moves the protein subunits relative to one another to achieve the necessary promoter remodeling that activates transcription.

The genes regulated by *soxRS* include many with obvious antioxidant functions, but the system also mediates general resistance to antibiotics. This resistance is mediated by *soxRS*-dependent changes in outer membrane proteins and by induction of the *acrAB* operon, which encodes a general efflux pump. Recent experiments demonstrate that *soxRS* has a role in the clinical development of antibiotic resistance. We characterized a clinical isolate of *Salmonella enterica* that developed quinolone resistance during treatment, and this strain had acquired a constitutive *soxR* mutation that contributed significantly to the strain's antibiotic resistance. We have also recently characterized quinolone-resistant *E. coli* from several clinical sources, and these strains also harbor *soxR*-constitutive alleles. Thus, *soxRS* has a central role in bacterial resistance to oxidative stress related to superoxide and nitric oxide, but this system also contributes to the development of clinically relevant antibiotic resistance.